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FBI pH 5.5	50-225 μ M [S]					AP1 pH 5.5	50-225 μ M [S]				
	Clone	Km	Vmax	Vmax/Km	X Improved		Clone	Km	Vmax	Vmax/Km	X Improved
50-225 μ M [S]	4F13 G12	62	15	0.242	7.1	50-225 μ M [S]	4F13 G12	365	44.2	0.121	6.1
	4F15 A11	58	13.6	0.234	6.9		4F15 A11	438	46	0.105	5.3
	4F15 C3	39	9	0.231	6.8		4F15 C3	563	27.6	0.049	2.5
	4F6 A11	191	28.3	0.148	4.4		4F6 A11	288	28.3	0.098	4.9
	4F3 B5	101.8	16.4	0.161	4.7		4F3 B5	378	25.3	0.067	3.4
	4F2 G10	41.2	9.4	0.228	6.7		4F2 G10	2209	61.3	0.028	1.4
	4F19 F2	235	59.5	0.253	7.4		4F19 F2	652	18	0.028	1.4
	4F21 C8	113	22	0.195	5.7		4F21 C8	305.5	55.9	0.183	9.2
	4F22 B2	161	21.5	0.134	3.9		4F22 B2	444	64.5	0.145	7.3
	4F28 G1	172	23.9	0.139	4.1		4F28 G1	ND	ND	ND	ND
22 hits (>3X Improved)						11 hits (>3X Improved)					
WT						WT					
349						450					
11.8						9.2					
0.034						0.02					
1						1					

Fig. 1

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Results: Kinetic parameters of pH-optimized candidates
H1 and B12 compared to wild type APAO

	FB1, pH 5.5					AP1, pH 5.5			
Vari- ant	kcat	km	kcat /km	Fold Impr	kcat	km	kcat /km	Fold Impr	
WT	150.0	98.0	1.5	1.0	240.0	430.0	0.6	1.0	
H1	2800.0	70.0	40.0	26.1	662.0	544.0	1.2	2.2	
B12	701.0	62.0	11.3	7.4	200.0	280.0	0.7	1.3	

Fig. 2

H1 and B12 share eight amino acid changes from wild type.

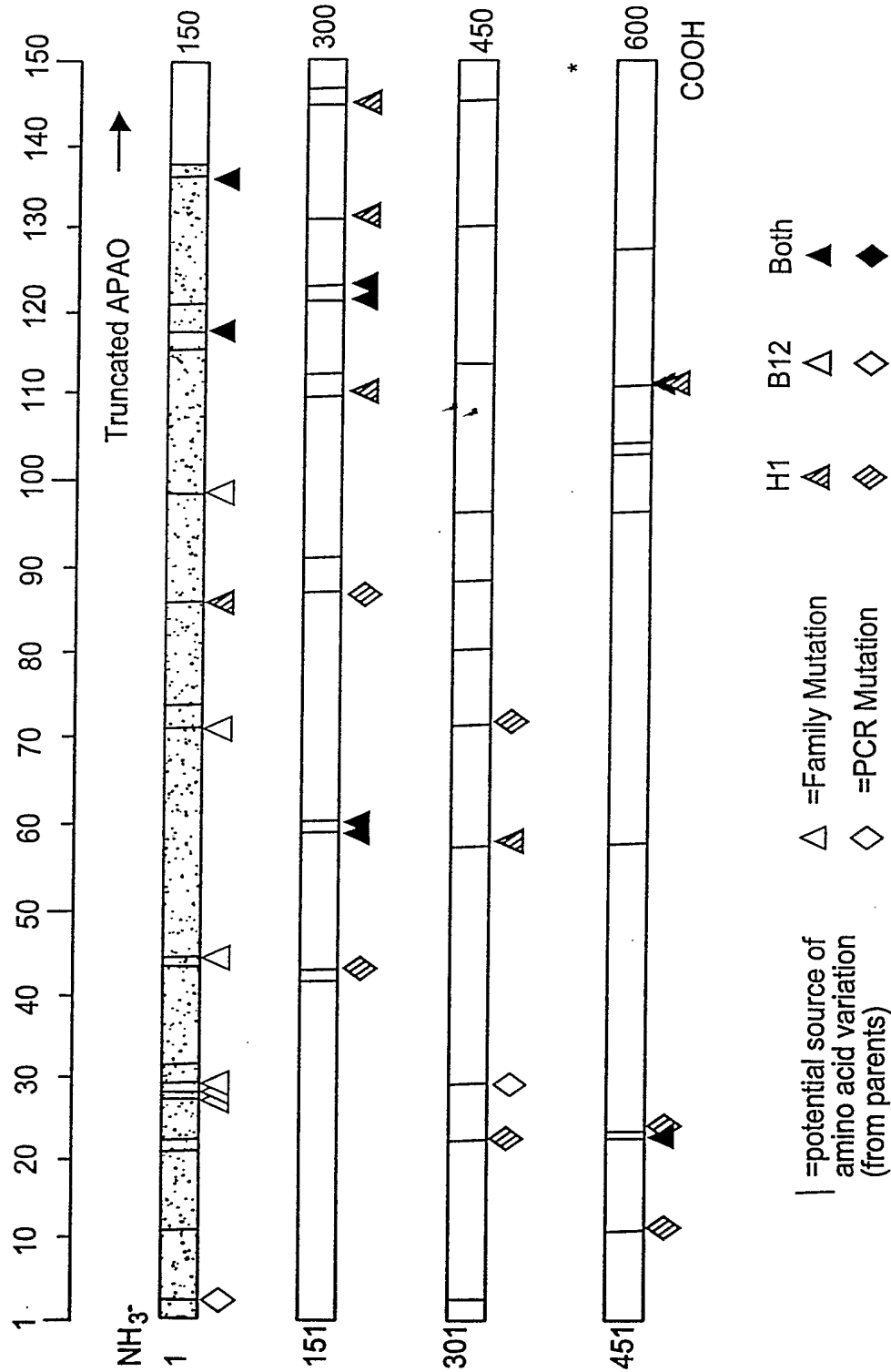
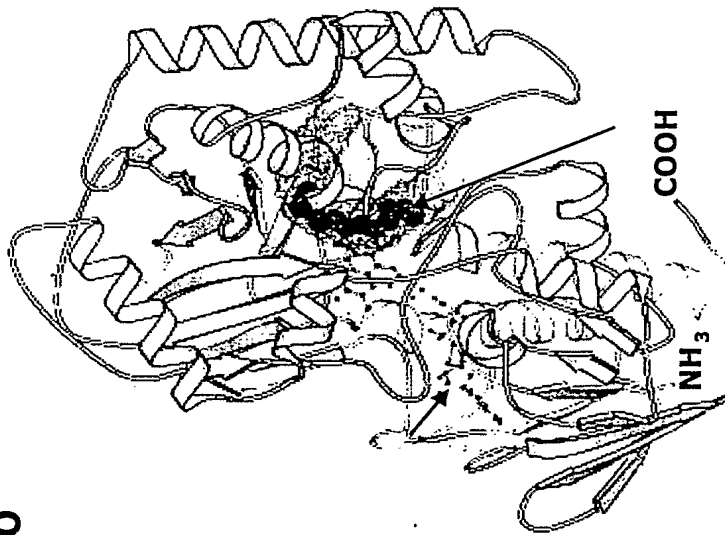


Fig. 3

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Several H1 & B12 mutations map to a putative substrate binding region of APAO

MPAO



Maize Polyamine Oxidase (MPAO)
30 Å crystal structure (Binda et al.
1999. Structure 7:265) Substrate
"tunnel" shown in wire form.

APAO



APAO (truncated, amino acids 142 -600)
3-D Model after Binda et al. Putative
substrate "tunnel" shown in center right.

Mutations: B12 \triangle \diamond H1 \triangle \diamond Both \blacktriangle \blacklozenge

Fig. 4

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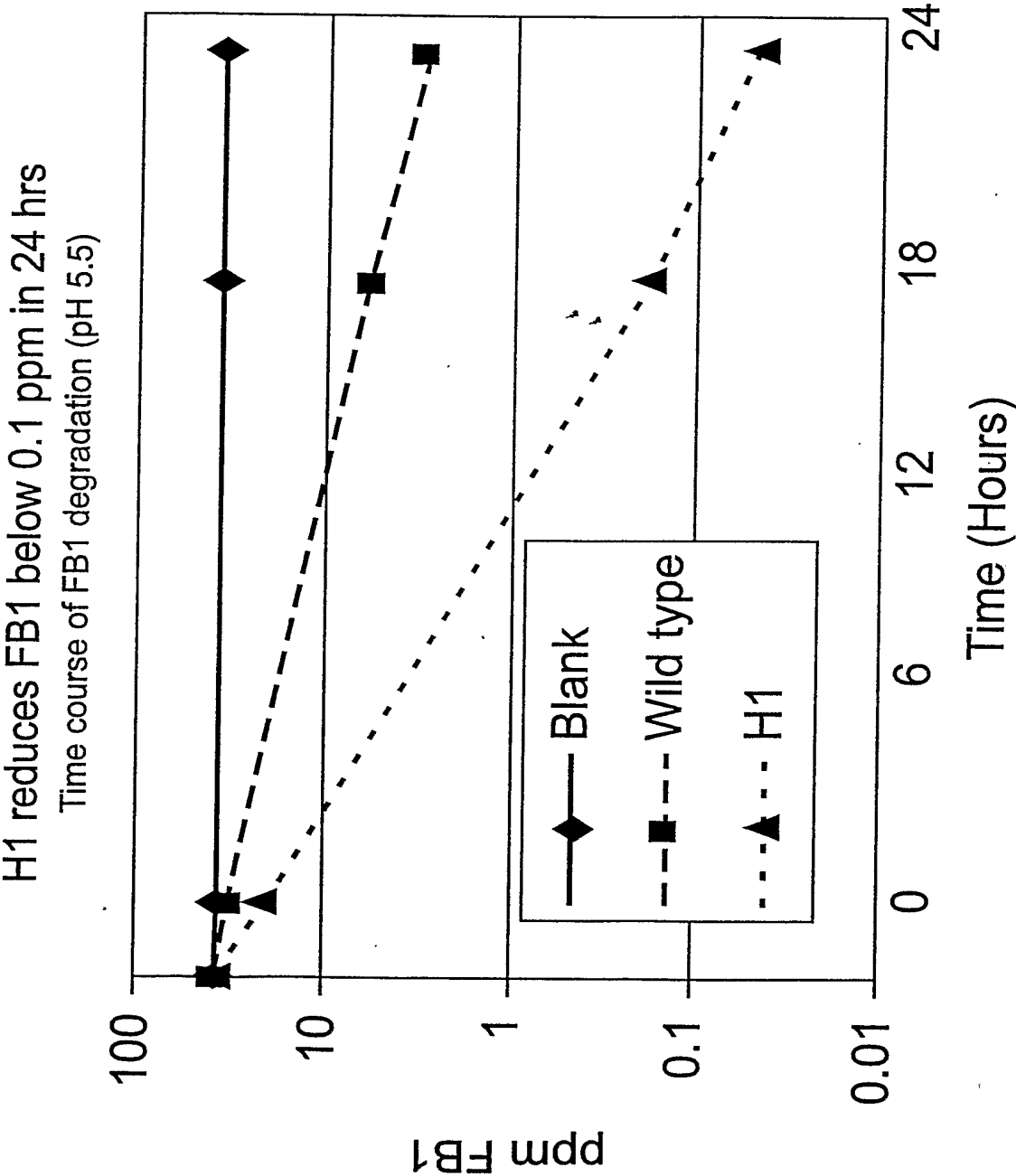


Fig. 5

H1 retains its high substrate specificity for fumonisins

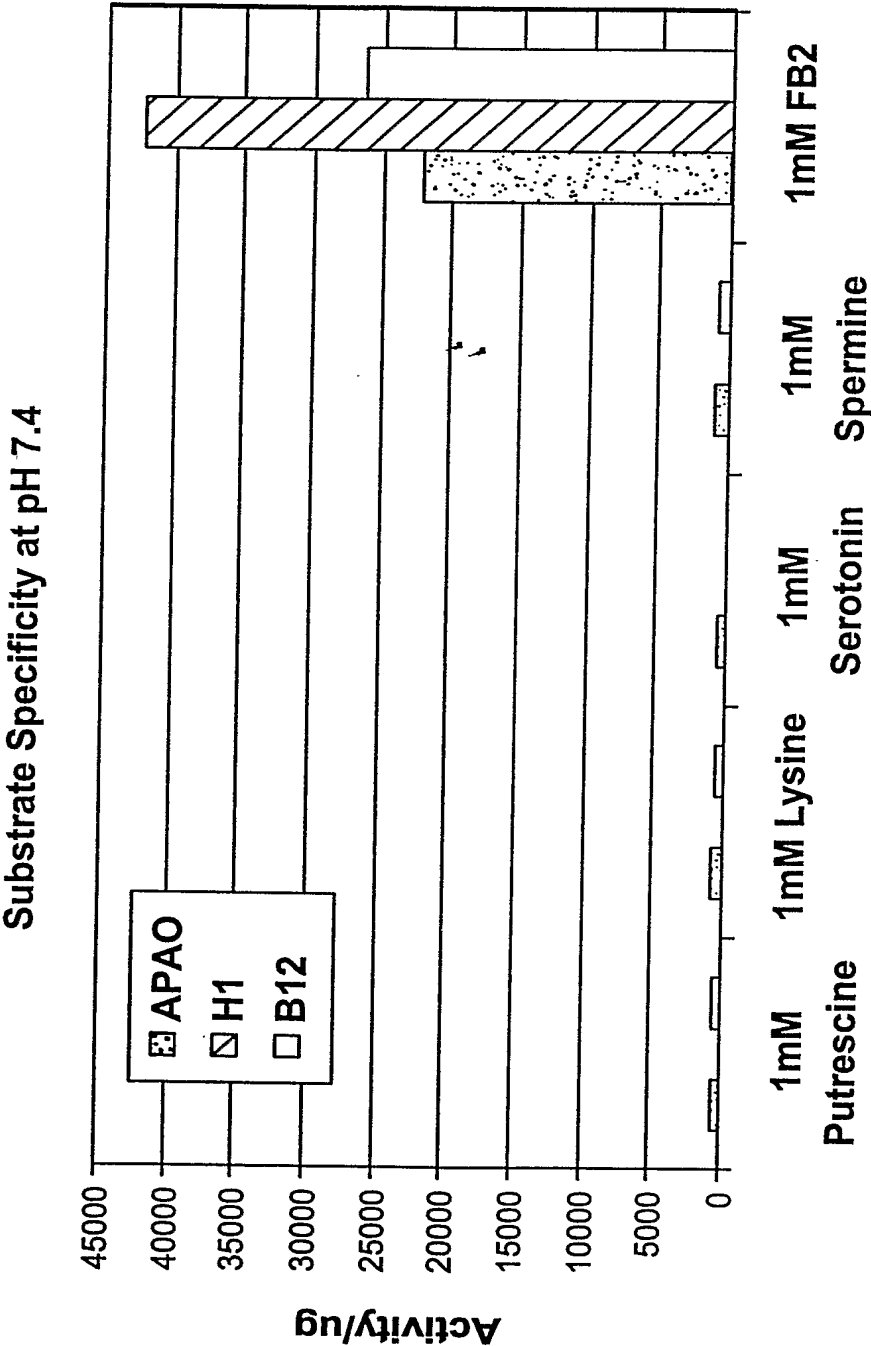
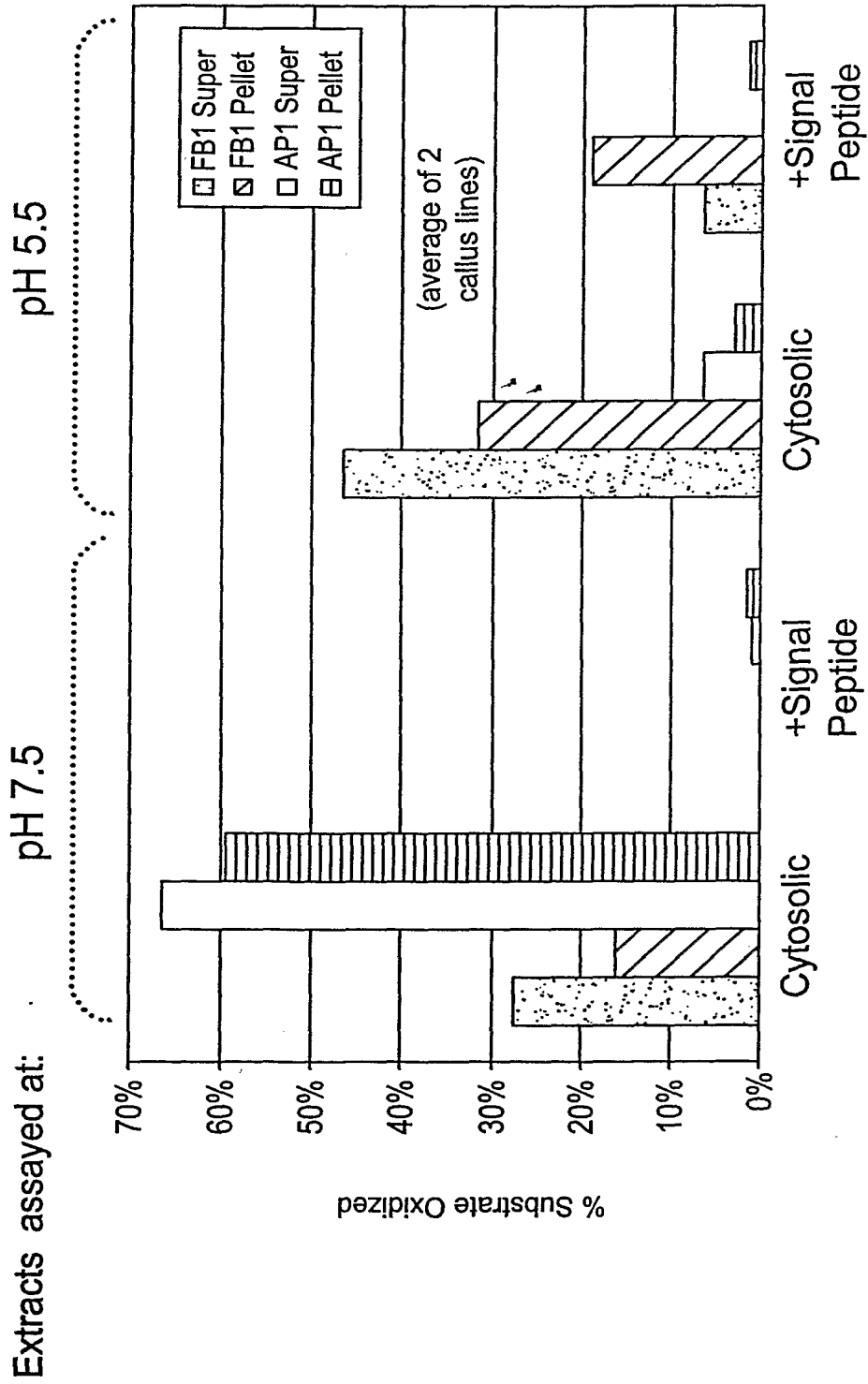


Fig. 6

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H1 is active in transgenic maize callus when expressed in cytosol or fused to a signal sequence



Wild type APAO H1- APAO

Fig. 7

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Fig. 8A

	rfu/min/ug (prior to preincubation)	%activity (after preincubation)
h1	1325	0.21
g6	1804	0.59
1b6	1612	0.55
1h8	1804	0.35
3e7	1558	0.71

Fig. 8B

	km	kcat
h1	259.12	2164.50
g6	154.19	1612.90

Fig. 8C

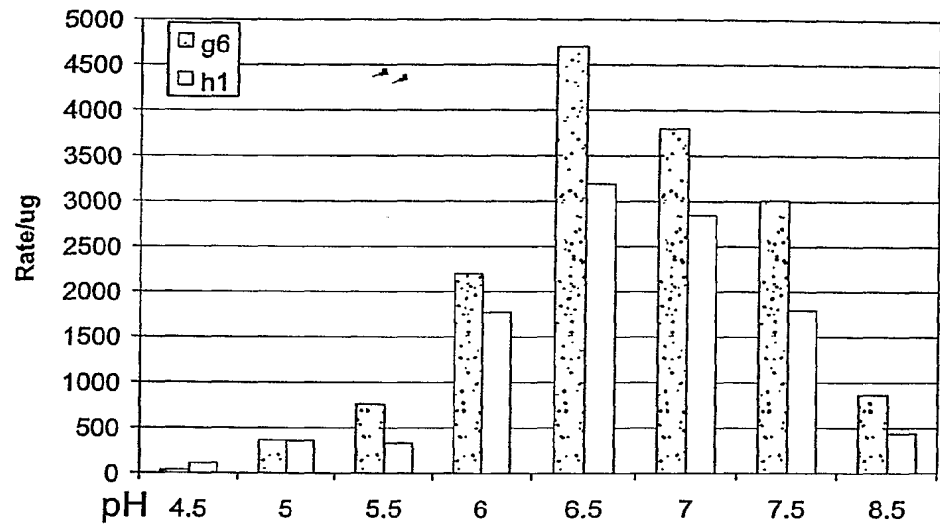
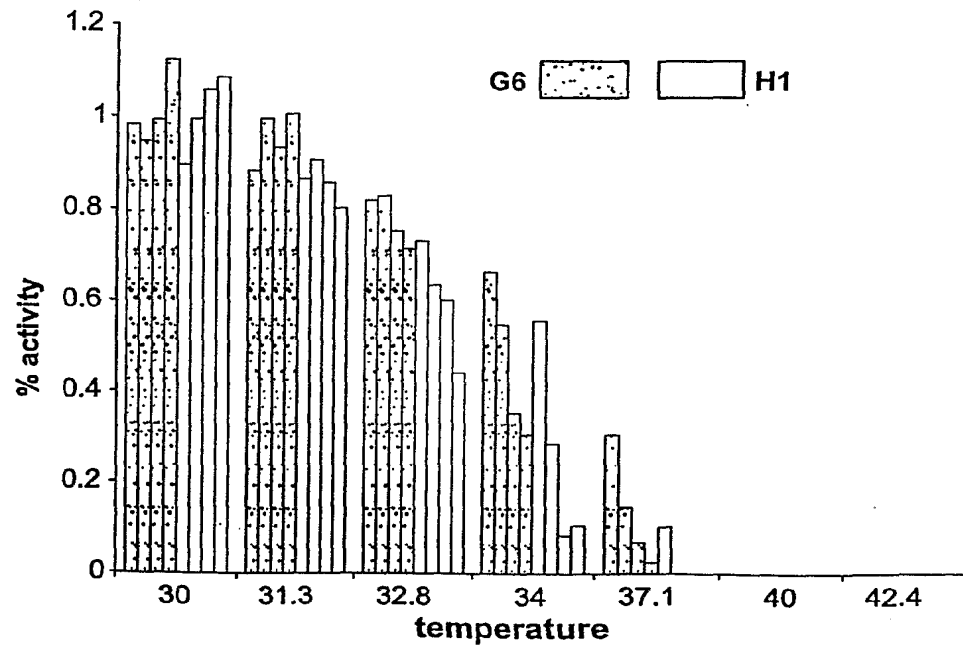


Fig. 8D



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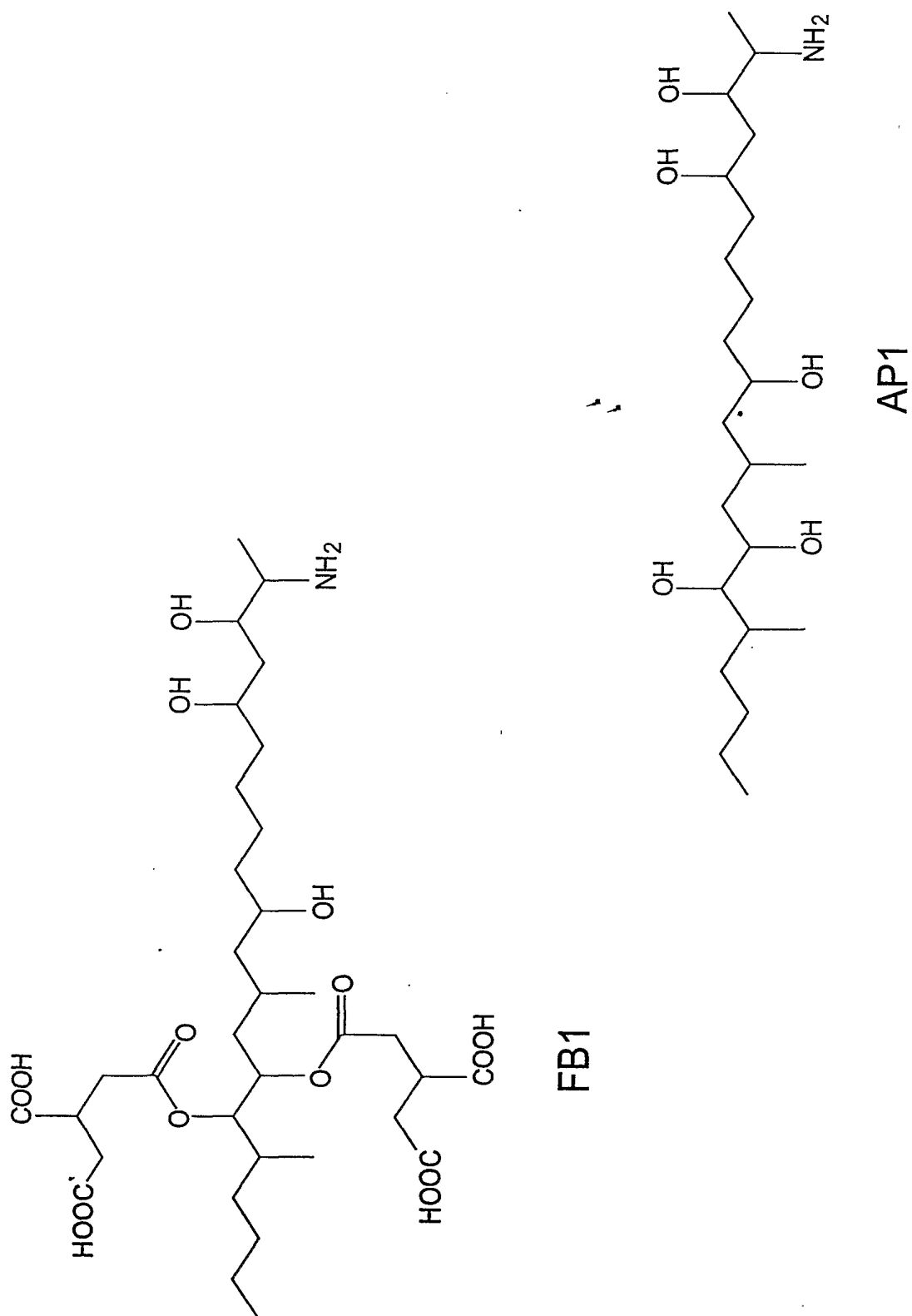


Fig. 9

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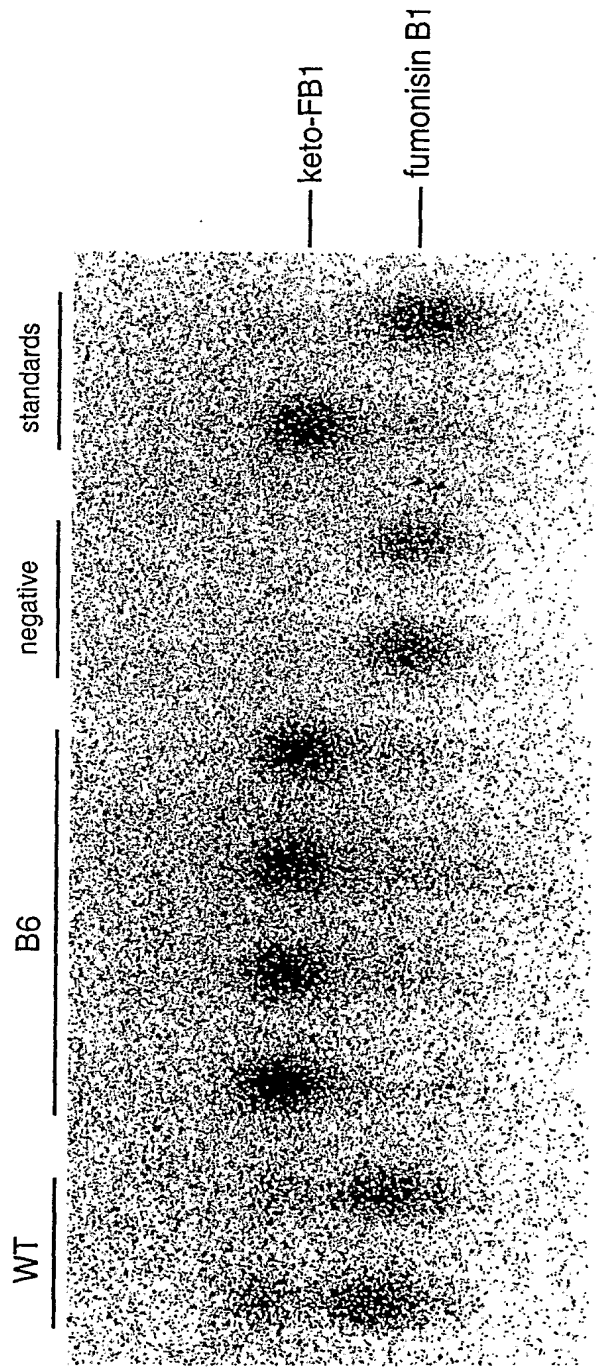


Fig. 10

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P18303
Ubi-BAA:APAO(B6) Glyc-

P18473
Ubi-APAO(B6) Glyc-

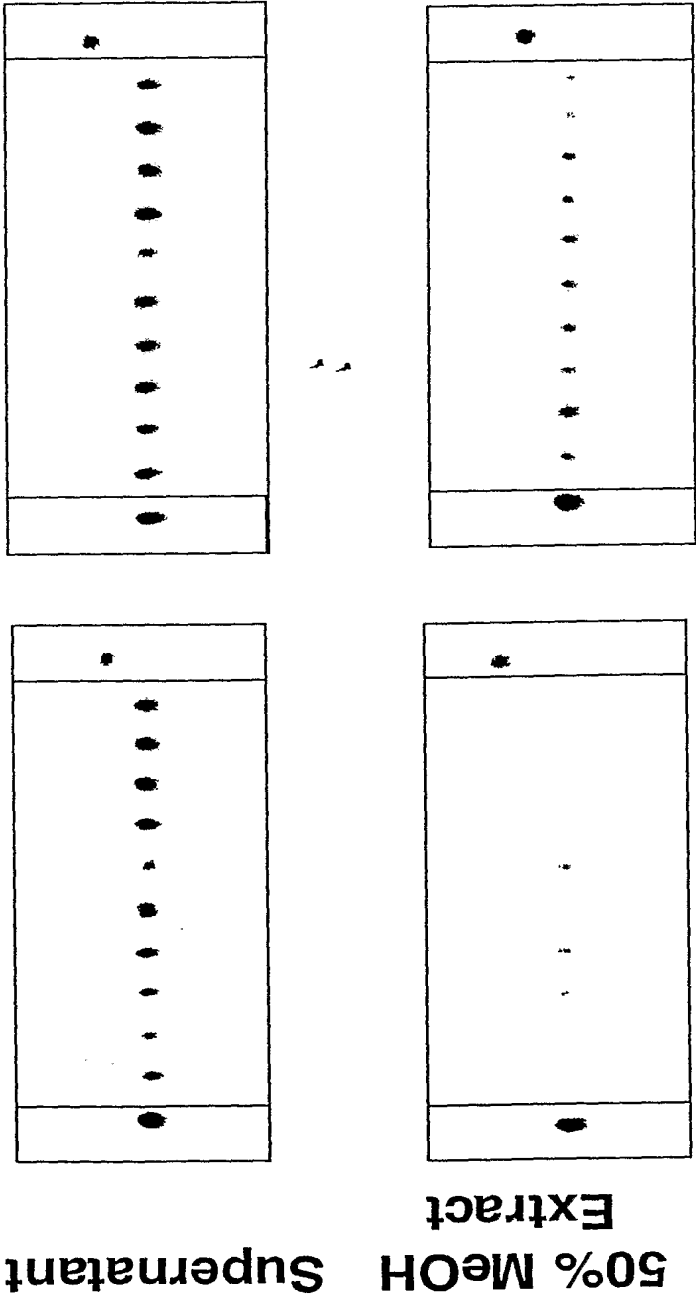


Fig. 11

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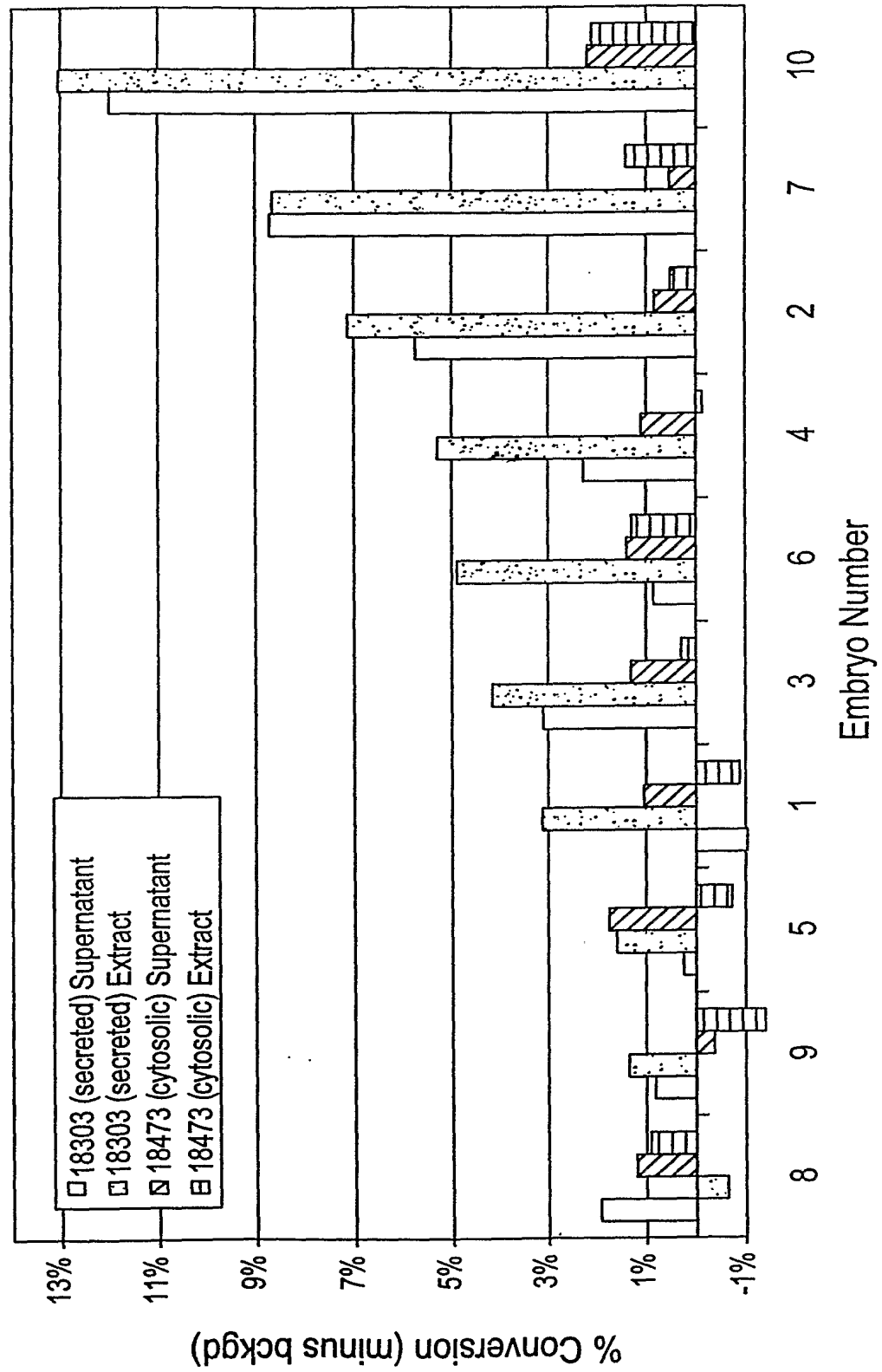


Fig. 12

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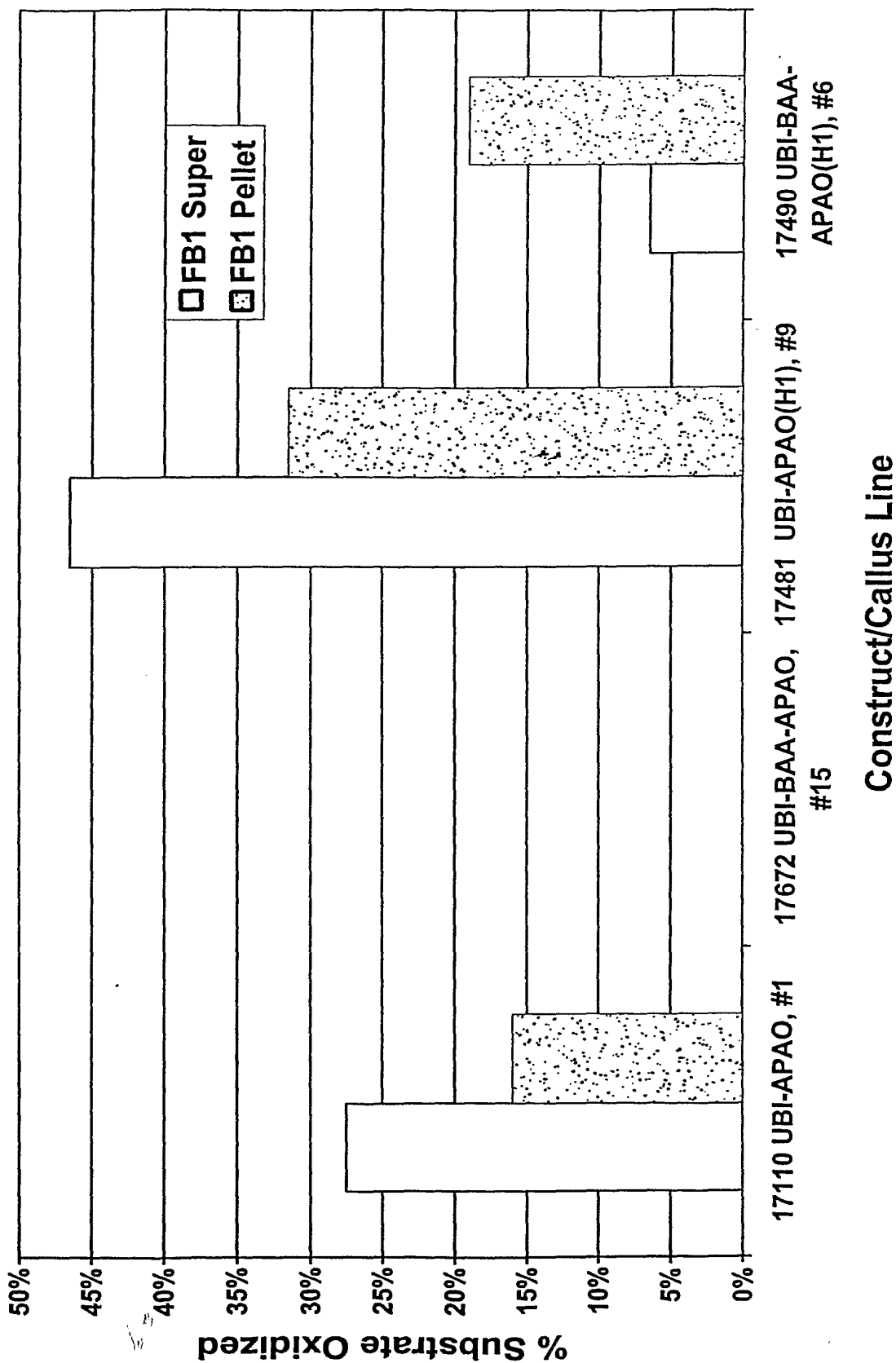


Fig. 13